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Published in:
Journal of Clinical Microbiology

Publication date:
1999

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1999, p. 3041–3043 Vol. 37, No. 9
0095-1137/99/$04.00+0

Evaluation of the Effects of Storage in Two Different Swab Fabrics and under Three Different Transport Conditions on Recovery of Aerobic and Anaerobic Bacteria

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Received 15 March 1999/Returned for modification 21 April 1999/Accepted 8 June 1999

Recovery of six anaerobic and five aerobic pathogens from viscose swabs and polyurethane swabs (Culturette EZ) was evaluated quantitatively, and transport in aerobic dry tubes, aerobic Amies transport medium (Transwab), and anaerobic universal transport medium (Port-a-Cul) was compared. The Culturette EZ in aerobic dry tubes gave the highest recovery levels. Data obtained with clinical specimens confirmed these results.

Recovery of bacteria from clinical specimens after transport or storage without the alteration of the relative proportions of the constituent species has long been recognized as a major problem (1–7).

Our goal was to select a transport medium that maintains the viability and relative proportions of all bacteria present in the patient material. We compared two types of swabs in presence or absence of different transport media. This led to six combinations: viscose swabs (V) (Copan, Italy) and polyurethane swabs (PU) (Becton Dickinson Microbiology Systems), in aerobic dry tubes (without transport medium [0]) (provided by Copan, Bovezzo, Italy, with the viscose swabs), aerobic Amies medium (A) (Medical Wire & Equipment Co. Ltd., Corsham, Wilts, United Kingdom), and anaerobic universal transport medium (Port-a-Cul [PAC]; Becton Dickinson Microbiology Systems). These combinations were inoculated with a standardized concentration of test strains. After various periods of incubation, the levels of recovery of the test strains were determined. Six anaerobic strains were used: *Clostridium innocuum*, *Fusobacterium necrophorum*, *Clostridium perfringens*, *Peptostreptococcus tetradius*, *Bacteroides fragilis*, and *Peptostreptococcus anaerobius*. Five aerobic strains were tested: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*.

Serial 10-fold dilutions of an overnight culture were prepared, depending on the strain, in brain heart infusion or thioglycolate broth with X and V factors. When needed, anaerobic conditions (8) were maintained by flushing the reduced broth under a continuing stream of CO_2 gas. Fifty microliters of each dilution was inoculated on solid plates (brucella-blood agar, blood agar, or chocolate agar plates); then aerobic strains were incubated for 24 h at 37°C and anaerobic strains were incubated for 48 h. The dilution that yielded 200 to 300 colonies per plate was used to inoculate the swabs. To imitate reality, 50 μl of the appropriate dilution ($\sim 5 \times 10^3$ CFU/ml) was pipetted into wells of a microplate and immediately absorbed with the swabs, which were then kept in a tube with or without transport medium. After intervals varying from 0 to 48 h of storage at room temperature, the swabs were rolled onto the surface of an agar medium appropriate for culturing of the various organisms. After incubation (24 h for the aerobic strains and 48 h for the anaerobic strains), the residual number of colonies was counted and compared with the original number. The latter was obtained when 50 μl of the appropriate dilution was inoculated immediately, without using swabs, onto the agar medium. All experiments were performed in duplicate.

The percentage of recovery of each strain was calculated by dividing the residual number of colonies by the original number and multiplying by 100.

Table 1 gives the median recovery levels determined for the aerobic and anaerobic strains at different times of incubation. The combination PU/0 results in the highest median recovery levels, a finding that is statistically significant by the Friedmann rank sum test. The combination V/A is the second best. For the other combinations, recovery levels are much lower. Between 24 and 48 h, recovery levels increased with the combinations V/A, PU/A, V/PAC, and PU/PAC, probably due to bacterial growth in the swabs. The median recovery levels for the combinations V/PAC and PU/PAC did not differ very much, although those for PU/PAC were somewhat lower than those for V/PAC. The combination V/0 results in the lowest recovery levels. At 24 h, some strains could not be cultured from the swabs (*N. gonorrhoeae* in V/0, V/PAC, or PU/PAC; *F. necrophorum* in V/0, PU/0, V/A, PU/A, or V/PAC; *P. anaerobius* in V/0, V/PAC, or PU/PAC; and *E. coli* in V/0) while others showed growth (*S. aureus* in V/0, PU/0, V/A, or PU/A; *E. coli* in all combinations except V/0; and *H. influenzae* in PU/0 only).

The finding that the recovery levels for PU/0 were higher than those for the other combinations was unexpected. Even with anaerobic strains, the PU/0 combination ranked first. This finding is surprising because the swab was not stored in a reduced environment. It might be explained by the nontoxic characteristics and sponge-like structure of the swab; the latter causes little desiccation, and the bacteria do not adhere tightly to the surface. The fact that the swab is not inserted into a medium reduces mechanical losses, since the swab is not squeezed by insertion into the agar. Despite the higher percentages of recovery in the first 12 h, the survival of the anaerobic strains stored in PU/0 after 24 and 48 h was in the same

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TABLE 1. Median percentages of recovery of anaerobic and aerobic strains tested on six different combinations of swabs and media

Storage time (h)	Median % recovery with the indicated swab fiber/transport medium combination					
	V/0	PU/0	V/A	PU/A	V/PAC	PU/PAC
For anaerobic strains (n = 6)[a]						
0	8.0	54	27.7	12.8	9.1	7.6
2	7.8	33.5	20.4	10	4.7	4.9
4	8.3	28.4	19.8	8.6	5.6	3.9
6	2.8	18.7	11.7	8.2	5	2.3
8	0.8	15.9	15.1	5.3	3.3	1.7
12	2.6	16.8	11.9	5.2	3.3	2.2
24	0.5	6.8	6.5	3	4.3	4.1
48	0	0.3	0.6	0.3	27.4	94.4
For aerobic strains (n = 5)[a]						
0	8.4	99.1	24.9	17.4	11.4	8.7
2	13	90.7	25.7	18.8	8.1	8.8
4	11	120.5	19.1	13.1	17.2	9.7
6	7.7	149.1	21.6	20.1	9	6
8	3.6	149	24.5	22.2	14	7.4
12[b]	1.4	43.1	15.4	15.3	3.2	6.7
24	0.3	NC[c]	22.5	52	10.7	16.1
48	0	NC	NC	NC	177.7	136.1

[a] See Table 2 for results for the individual aerobic and anaerobic strains.
[b] Recovery was determined for three of five strains: the *N. gonorrhoeae*, *H. influenzae*, and *S. pneumoniae* strains.
[c] NC, not countable.

range as that obtained with V/A. This is probably due to the effect of oxygen on the anaerobic bacteria. The transport medium PAC, compared in earlier studies with Accu-CulShure (2) and Anaerobic Specimen Collector (6) on clinical specimens, gave lower recovery levels than PU/0 in our studies. The use of a transport medium does not improve the performance of PU. This effect can be seen by comparing the combinations PU/0, PU/A (5% agar), and PU/PAC (10% agar).

It is not surprising that the median percentages of recovery with the dry viscose swab (V/0) were low for all strains tested (8.2 to 0%). It has been shown (5) that bacteria, which adhere to polar dry fibers, become desiccated, and only 3 to 5% can be recovered. The percentages of recovery, of the different bacterial strains at time zero are given in Table 2. It illustrates the loss of organisms due to adhesion to the swab material. For the aerobic strains the lowest adhesion is shown for the PU/0 combination. For *P. tetradius*, *P. anaerobius*, *C. perfringens*, *C. innocuum*, and *F. necrophorum*, PU/0 shows the lowest adhesion. For *B. fragilis*, V/A gave a slightly higher level of recovery than PU/0. These results indicate that the higher percentages of recovery with PU/0 upon transport are at least partly due to less adhesion of organisms to the swab material.

TABLE 2. Recovery levels of individual anaerobic and aerobic strains tested on six different combinations of swabs and media at time zero[a]

Strain	% Recovery with the indicated swab fiber/transport medium combination					
	V/0	PU/0	V/A	PU/A	V/PAC	PU/PAC
Anaerobic						
C. innocuum	4.0	53.9	16.6	6.9	10.5	4.6
F. necrophorum	1.6	33.5	13.2	32.4	0.7	4.4
C. perfringens	15.5	54.2	28.1	14.2	12.8	10.9
P. tetradius	12.4	68.8	32.0	6.7	7.9	9.2
B. fragilis	1.2	23.9	27.2	11.5	5.0	6.7
P. anaerobius	42.5	101.3	34.0	18.9	10.4	8.6
Aerobic						
E. coli	15.4	112.3	38.8	21.4	11.3	8.2
S. aureus	7.2	81.5	24.9	15.4	7.5	5.1
S. pneumoniae	2.7	99.1	15.9	10.7	11.9	15.5
N. gonorrhoeae	8.4	101.8	16.2	17.4	11.4	8.7
H. influenzae	21.0	95.4	48.4	27.3	19.1	23.0

[a] Swabs were taken and were inoculated without delay on plates.

TABLE 3. Recovery of strains from clinical specimens[a] with PU/0 or V/A

Bacteria	No. of organisms detected by using:				
	Inoculating loop (0 h)	PU/0		V/A	
		0 h	24 h	0 h	24 h
Aerobic gram-positive cocci	20	33	22	23	19
Aerobic gram-negative cocci			1		
Aerobic gram-positive rods	3	2	1	1	1
Aerobic gram-negative rods	13	18	19	16	16
Anaerobic gram-positive cocci	1	1	1	1	
Anaerobic gram-negative rods	1	1	2	1	
Yeasts	6	5	6	4	2
Total no. of strains (n = 82)	44	60	52	46	39
Total no. of species (n = 32)	23	25	23	21	32

[a] Pus samples (n = 30) were inoculated immediately (t = 0 h; inoculating loops) and after 24 h of storage at 4°C.

In addition, the performances of PU/0 and V/A were investigated with clinical specimens. A total of 30 pus samples obtained from 27 different patients by drainage of abscesses were used; they included specimens of intra-abdominal origin (n = 11), specimens from the neck (n = 1), the sinus maxillaris (n = 3), and the thorax (n = 7), and 8 specimens of unknown origin. The samples were inoculated semiquantitatively (by inoculating loops) on selective or nonselective plates and were vortexed in broth. From the same samples, swabs were taken and cultured in the same way after 0 and 24 h of storage at 4°C. The results (Table 3) obtained with PU/0 were superior to those obtained with V/A. In total, 82 strains (32 species) were isolated. At 0 h of incubation, 60 of the 82 strains were isolated with PU/0 versus 46 of the 82 strains with V/A. When the inoculation loops were used, 44 strains were isolated. After 24 h of storage, 52 and 39 strains were isolated from PU/0 and V/A, respectively. With PU/0 an additional 27 strains were isolated, which were not isolated by using V/A or inoculation loops. When V/A or the inoculation loops were used, three or six additional strains were isolated, respectively, which were not cultured from PU/0.

We conclude that the combination PU/0 yields the highest recovery levels during the first 24 h. Our experiment shows that the relative proportions of bacteria are severely disturbed when swabs are not processed within 24 h after sampling, since some strains die while others show growth.

REFERENCES

1. **Alfa, M., and A. Lee.** 1982. A transport method for swab specimens submitted for aerobic and anaerobic bacteriology. J. Clin. Pathol. **35:**555–560.
2. **Baron, E. J., M. L. Vaisanen, M. McTeague, C. A. Strong, D. Norman, and S. M. Finegold.** 1993. Comparison of the Accu-CulShure system and a swab placed in a B-D Port-a-Cul tube for specimen collection and transport. Clin. Infect. Dis. **16**(Suppl. 4)**:**S325–S327.
3. **Brook, I.** 1987. Comparison of two transport systems for recovery of aerobic

and anaerobic bacteria from abscesses. J. Clin. Microbiol. **25:**2020–2022.
4. **Collee, J. G.** 1980. Factors contributing to loss of anaerobic bacteria in transit from the patient to the laboratory. Infection **8**(Suppl. 2)**:**S145–S147.
5. **Collee, J. G., B. Watt, R. Brown, and S. Johnstone.** 1974. The recovery of anaerobic bacteria from swabs. J. Hyg. **72:**339–347.
6. **Johnson, S., F. Lebahn, L. R. Peterson, and D. N. Gerding.** 1995. Use of an anaerobic collection and transport swab device to recover anaerobic bacteria from infected foot ulcers in diabetics. Clin. Infect. Dis. **20**(Suppl. 2)**:**S289–S290.
7. **Perry, J. L.** 1997. Assessment of swab transport systems for aerobic and anaerobic organism recovery. J. Clin. Microbiol. **35:**1269–1271.
8. **Summanen, P., E. J. Baron, D. Citron, C. A. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual. Star Publishing Company, Belmont, Calif.